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The Identity and Prevalence of Trypanosomes in White-tailed Deer (*Odocoileus virginianus*) from Southern Florida

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ABSTRACT: *Trypanosoma cervi* Kingston and Morton, 1975, is reported from white-tailed deer, *Odocoileus virginianus* (Zimmermann), in Collier, Dade, and Monroe counties of southern Florida. Annual prevalences from thin blood smears averaged 21% (range 7–41%) from 1984 to 1989. Fall prevalence averaged 93% (range 85–100%) when determined by culture of blood in a monophasic liquid medium, whereas examination of thin films averaged only 18% in these years. Examination of thick blood smears proved comparably effective when compared to blood culture, resulting in the detection of 98% of infections diagnosed by blood culture.

KEY WORDS: *Trypanosoma cervi*, white-tailed deer, *Odocoileus virginianus*, prevalence, diagnostic techniques, Florida.

The trypanosome of white-tailed deer in the United States has been identified as *Trypanosoma cervi* Kingston and Morton, 1975. Infected deer have been found from central Florida north to West Virginia and west to Arkansas (Kingston and Crum, 1977; Davidson et al., 1983). However, no data are available on the prevalence of this parasite in deer populations from the southernmost portion of the range in Florida.

Since 1984 we have participated in an ongoing cooperative study of white-tailed deer populations in southern Florida with the Florida Game and Fresh Water Fish Commission (FGFWFC) and the National Park Service (NPS). Trypanosomes were found often in high prevalence and this study describes the identity and prevalence of the trypanosomes as determined by examination of blood films and blood cultures.

Materials and Methods

Blood samples were obtained from a total of 238 white-tailed deer collected during 1984–1989 in Collier, Dade, and Monroe counties, Florida. Deer samples comprised 50 males and 188 females, representing 39 fawns (age 3–11 mo), 51 yearlings (12–23 mo), and 148 adults (over 23 mo). Between October 1984 and October 1986, 9–25 deer were sampled during each of 3 seasons: spring (March), summer (June–August), and fall (October–November). From 1987 to 1989, 27–50 deer were obtained during fall only. Cardiac blood samples were taken immediately after the deer were killed by gunshot at night by FGFWFC or NPS biologists and were refrigerated in anticoagulant tubes (EDTA or sodium citrate) until the following morning. Several thin blood films were prepared from whole blood of each deer, air-dried, and fixed in absolute methanol. Additionally, in 1989 standard thick films consisting of a circular drop of blood 5–8 mm in diameter and thin films of blood centrifuged for hematocrit determinations were prepared. These latter films, obtained from a limited number of deer, made both from the vicinity of the buffy coat and from the packed end of the hematocrit tube, were fixed and stained as standard thin films. All 1989 thin films were stained on the day of preparation for 1 hr with Giemsa (Harleco, EM Diagnostic Systems, Inc., Gibbstown, New Jersey) at a dilution of 1:9, pH of 7.0 or 7.2. Thick films were air-dried for at least 24 hr and then placed face side down for 1 hr in a dish containing dilute Giemsa stain (1:20), which hemolyzed the erythrocytes. Slides were rinsed gently with distilled or bottled drinking water and air-dried. An additional thin film from each deer was stained by the Leukostat procedure (Fisher Scientific, Orangeburg, New York), which gave results comparable to Giemsa staining when done on the day of preparation. The thin blood films collected from 1984 to 1988 were generally poorly stained, and most required destaining and restaining when unstained duplicates were not available. Poorly stained films were destained for 10 min in 95% acid-ethanol, neutralized by immersion in 95% alkaline-ethanol, and washed for a few minutes in running tap water. Old, unstained and newly destained thin films were then immersed in Giemsa stain in an acetone–methanol–distilled water solution buffered to pH 6.4 for 2 hr, using an unpublished procedure developed by R. B. Kimsey, University of California, Davis. This generally gave results comparable to material stained when fresh. All smears were screened for trypanosomes at 160×, covering the entire smear with special attention paid to the "tails" and edges of the films. In infections of high intensity, trypanosomes could be found anywhere on the film, but in infections of low intensity they were most often on the periphery. A 5-ml sample of blood from each deer collected in October 1985, 1986, 1988, and 1989 was inoculated into 2–5 ml of a monophasic liquid medium (Sadigursky and Brodskyn, 1986). The cultures, maintained at room temperature, were examined 5–7 days following inoculation, and in the case of ap-

Table 1. A comparison of mensural values for trypanosomes of white-tailed deer from southern Florida with those of *Trypanosoma cervi*.

Source	Measurements in μm							
	PK	KN	PN	NA	BL	FF	L	W
S. Florida								
Mean	12.6	7.6	20.2	28.6	47.8	6.1	53.9	9.4
Range	6–22	5–16	13–31	19–39	32–65	0–12	39–76	4–14
SE USA*								
Mean	13.5	8.3	22.0	26.3	48.2	9.5	57.5	5.6
Range	5–27	2–14	14–34	12–41	31–74	3–21	40–83	3–15
Type host (elk)†								
Mean	12.2	7.0	19.4	24.8	45.4	6.6	52.0	4.6
Range	5–20	4–9	11–32	20–30	32–56	3–11	40–61	3–8

* From Kingston and Crum (1977).
† From Kingston and Morton (1975).

parent negatives, several times for up to 3 mo thereafter. Thin films of the culture forms were prepared from 3 positive samples collected in October 1989, and fixed and stained similarly to thin blood films.

A series of 25 trypomastigotes with clear diagnostic characters were selected from slides collected from 1986 to 1989. These were measured by calibrated ocular micrometer at 1,000× under oil immersion. Taxonomic characters utilized were those of Kingston and Morton (1975) and are abbreviated in Tables 1 and 2 as follows: PK, posterior end to kinetoplast; KN, nucleus to kinetoplast; PN, posterior end to nucleus; NA, nucleus to anterior end; BL, total length minus free flagellum; FF, free flagellum; L, total length; W, width; NI, nuclear index, PN/NA; and KI, kinetoplast index, PN/KN. Nucleus location was measured to the center of the nucleus, while width refers to the greatest breadth of the trypanosome exclusive of the undulating membrane. This latter character may differ in its measurement by other authors. The free flagellum was not measured unless its entire length was evident. All trypanosomes measured were trypomastigote forms and are compared below only with bloodstream trypomastigote data of other authors. Chi-square tests were used to compare prevalences. Representative blood films have been deposited in the U.S. National Parasite Collection, Beltsville, Maryland (accession nos. 81000–81002).

Results

Identity of the trypanosomes

Comparison of mensural data of the trypanosomes from the population of deer in southern Florida (Table 1) with those published by Kingston and Morton (1975) and Kingston and Crum (1977) demonstrated clearly that they were conspecific with *Trypanosoma cervi,* and distinct from *Trypanosoma theileri* (Table 2). In *T. cervi* there was a smaller ratio of the distance from the posterior end–nucleus to the kinetoplast–nucleus distance (KI), a lower mean ratio between the distance posterior end–kinetoplast to body length (PK/BL), and a greater mean ratio of the nucleus–anterior end to the body length (NA/BL). Given the difference in measurement technique, i.e., the use of an ocular micrometer (present study) instead of projected photomicrographs measured with a calibrated map reader, the concordance of the data was remarkable. Only the mean width measurement (W) differed appreciably, and this was probably a combination of fixation or configuration artifacts and the inclusion of both slender and broad forms (Kingston and Crum, 1977) in the sample. Three trypanosomes of the measured 25 could be described as long, slender forms; the remainder of the sample was broad in appearance. The critical diagnostic characters of the southern Florida sample (Table 2) either were completely within the range of variation described for *T. cervi* or overlapped at one end of the range. No attempt was made to measure bloodstream or cultural epimastigote forms because of their variability in shape. The ratio of trypomastigote (T) to epimastigote (E) forms indicated highly significant ($P < 0.0001$) seasonal differences: in summer 1985, 6 T : 31 E; in pooled fall samples from 1984 to 1988, 25 T : 6 E; and pooled samples from 5 deer in fall 1989, 53 T : 2 E.

Prevalence

Thin films were the only samples available for every sampling period (Table 3). Using only the data obtained from those films, we found significant differences between the prevalence of

Table 2. A comparison of diagnostic characters for trypanosomes of white-tailed deer from southern Florida with those of *Trypanosoma cervi* and *Trypanosoma theileri*.

Source	FF:BL	NI	KI	PK/BL	NA/BL
			T. cervi		
S. Florida					
Mean	1:6.8	0.71	2.77	0.26	0.60
Range	4.8–12.6	0.5–1.1	1.9–4.6	0.2–0.4	0.5–0.9
SE USA*					
Mean	1:5.7	0.88	2.86	0.28‡	0.55‡
Type host (elk)†					
Mean	1:6.9	0.78	2.77	0.27	0.55
Range	—	0.5–1.3	2.8–3.6	0.2–0.4	0.5–0.6
			T. theileri§		
Broad forms					
Mean	1:8.5	0.91	5.68	0.39	0.51
Range	7.0–9.3	0.7–1.1	4.4–5.0	0.3–0.5	0.5–0.6
Slender forms					
Mean	1:8.6	0.84	4.00	0.35	0.52
Range	6.3–13.8	0.5–1.1	3.6–4.1	0.2–0.5	0.5–0.6

* From Kingston and Crum (1977).
† From Kingston and Morton (1975).
‡ Calculated from data of Kingston and Crum (1977).
§ Saisawa et al. (1933), quoted by Kingston and Morton (1975).

trypanosomes during fall of 1984 (44%) and spring of 1985 (0%, $P = 0.04$), between spring of 1985 (0%) and summer of 1985 (56%, $P = 0.04$), and between summer of 1985 (56%) and summer of 1986 (8%, $P = 0.05$). There were no significant differences ($P = 0.15$) when all 3 summer samples were compared with each other. Spring samples from 1985 (0%) and 1986 (15%) did not differ significantly ($P = 0.50$), and there were no differences among the samples collected in fall of each year ($P = 0.37$). The seasonal and annual prevalence of *T. cervi* in southern Florida, as determined from the examination of thin blood films (Table 3), was likely a considerable underestimate as suggested by the results of blood culture (Table 4). In the fall of 1989, when thick films and centrifuged thin films were prepared, 4 diagnostic methods were employed. Both thick films and culture methods were very sensitive for detecting infection by *T. cervi* and resulted in prevalences of 98 and 100% in comparison to 16 and 15%, respectively, detected by normal and centrifuged thin blood films. The latter did not prove to be better for the detection of try-

Table 3. Seasonal and annual prevalences of *Trypanosoma cervi* determined from thin blood films of white-tailed deer from southern Florida, 1984–1989.

	Spring (Mar.)			Summer (June–Aug.)			Fall (Oct.–Nov.)			Totals		
Year	No. deer exam.	% pos.	95% C.I.	No. deer exam.	% pos.	95% C.I.	No. deer exam.	% pos.	95% C.I.	No. deer exam.	% pos.	95% C.I.
1984	0	—	—	13	38	16–68	9	44	17–76	22	41	21–64
1985	12	0	0–30	25	56	35–75	15	20	6–49	52	33	20–48
1986	20	15	5–40	12	8	1–40	14	7	1–39	46	11	4–25
1987	0	—	—	0	—	—	27	7	2–26	27	7	2–26
1988	0	—	—	0	—	—	41	22	11–39	41	22	11–39
1989	0	—	—	0	—	—	50	16	8–31	50	16	8–31
Totals	32	9	3–27	50	40	26–56	156	17	12–25	238	21	16–28

Table 4. Prevalences of *Trypanosoma cervi* in white-tailed deer in southern Florida during October as determined by thin blood films vs. blood culture techniques.

Year	No. deer examined	% positive by	
		Thin blood film	Blood culture
1985	15	20	93
1986	14	7	92
1988	41	22	85
1989	50	16	100*
Totals	120	18	93

* This value represents results of cultures from 46 deer; cultures from 4 other deer were contaminated.

panosome infection than thin films of uncentrifuged whole blood. More importantly, the use of thick films can provide an adequate survey method when cultures cannot be used, as they resulted in the diagnosis of 98% of the 46 infections found by culture of fresh blood and all 4 of the positive samples for which the cultures were contaminated. By using thin films or centrifuged blood sample techniques, only 15–16% of the positive samples were detected, which proved to be highly significant ($P < 0.001$) from either thick film or culture methods.

Discussion

The identification of *T. cervi* from white-tailed deer in southern Florida was not surprising given the broad distribution of this parasite in North American cervids (Kingston and Morton, 1975). Because deer in southern Florida intermingle freely with range cattle in the Collier County study area, it was possible that infection by *Trypanosoma theileri,* which is known in Florida (Ristic and Trager, 1958), might occur in the deer. However, none of the trypanosomes found on blood smears was *T. theileri. Trypanosoma cervi* appears to be host specific to cervids, as suggested by the failure of Kingston and Morton (1975) to infect cattle with *T. cervi* from elk. It is unlikely that deer are infected from transmission of *T. theileri* among sympatric cattle herds.

In 1985 the very high prevalence (56%) of trypanosomes on thin blood films from the summer samples (June and August) indicates that transmission probably occurred following the March sample, when no infections were found. Ten of 14 (71%) June–August infections had bloodstream epimastigotes present, while only 5 had trypomastigotes. The ratio of trypomastigote to epimastigote forms in this summer sample, when compared to pooled fall samples from 1984 to 1988 and the 1989 fall sample, also supports summer transmission of *T. cervi* in southern Florida. Epimastigotes are the multiplicative stage of *T. cervi* in the bloodstream and in culture (Kingston and Morton, 1975; Kingston and Crum, 1977). The presence of epimastigotes in numbers greater than those of trypomastigotes should indicate either a recently acquired active infection or recrudescence of a chronic infection. In the related *T. theileri,* reproduction also occurs in the epimastigote stage, and when infection begins, fairly high parasitemias may be apparent (Hoare, 1972). After 2–4 wk, parasitemias cannot be detected by microscopic examination of blood films, but the blood remains infective for up to 1 yr. There is no lasting immunity. In the temperate zone, reinfection may occur in spring when horse-flies again appear. The available data for *T. cervi* suggest similar epidemiology. In 1986, prevalence of *T. cervi* in spring was twice that of the subsequent summer and fall. This could reflect annual variation in the seasonal abundance of the presumed tabanid fly vectors (Kingston, 1981), with tabanid appearance earlier in spring that year than usual. Thirty-five of the 118 species of tabanid flies known from Florida (Jones and Anthony, 1964) are considered to be economically important on the basis of abundance, and 26 of these reach peak densities between April and July, the period during which increased prevalence of *T. cervi* and epimastigotes was found. The small sample from the summer of 1986 ($N = 12$) may have been partially responsible for the discrepant prevalence pattern observed that year. The October samples for 1985–1989 show prevalences, based on thin blood smears, varying from 7% to 22%, and by culture from 86% to 100%. Utilizing thin blood films, Davidson et al. (1983) found maximum prevalence (61%) of *T. cervi* from July to September, with a drop to one-half that level from October to December (30%). They also reported the highest prevalence of epimastigote forms on blood films taken from July to September. Their samples came from central Florida north to West Virginia and Arkansas, where the climatic pattern differs considerably from subtropical southern Florida. Our data are consistent with those of Davidson et al. (1983) and suggest that the relatively insensitive thin blood film technique

may provide better evidence of fluctuations in intensity due to transmission and infection phase than do the highly sensitive methods of culture or thick blood films. The prevalence of *T. cervi* appears to be high wherever adequate samples have been examined (Kistner and Hanson, 1969; Stuht, 1975; Davidson et al., 1983).

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